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PTEN loss and chromosome 8 alterations in Gleason grade 3 prostate cancer cores predicts the presence of un-sampled grade 4 tumor: implications for Active Surveillance

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Abstract

Men who enter active surveillance because their biopsy exhibits only Gleason grade 3 (G3) frequently have higher grade tumor missed by biopsy. Thus, biomarkers are needed that, when measured on G3 tissue, can predict the presence of higher grade tumor in the whole prostate. We evaluated whether PTEN loss, chromosome 8q gain (*MYC*) and/or 8p loss (*LPL*) *measured only on G3 cores* is associated with un-sampled G4 tumor. A tissue microarray was constructed of prostatectomy tissue from patients whose prostates exhibited only Gleason score 3+3, only 3+4, or only 4+3 tumor (n=50 per group). Cores sampled only from areas of G3 were evaluated for PTEN loss by immunohistochemistry, and *PTEN* deletion, *LPL*/8p loss, and *MYC*/8q gain by fluorescence *in situ* hybridization (FISH). Biomarker results were compared between Gleason score 6 vs. 7 tumors using conditional logistic regression.

PTEN protein loss, odds ratio=4.99, p=.033, *MYC*/8q gain, odds ratio=5.36, p=.010, and *LPL*/8p loss, odds ratio=3.96, p=.003 were significantly more common in G3 cores derived from Gleason 7 vs. Gleason 6 tumors. *PTEN* gene deletion was not statistically significant. Associations were stronger comparing Gleason 4+3 vs. 6 than for Gleason 3+4 vs. 6. *MYC*/8q gain, *LPL*/8p loss, and PTEN protein loss measured in G3 tissue microarray cores strongly differentiate whether the core comes from a Gleason 6 or Gleason 7 tumor. If validated to predict upgrading from G3 biopsy to prostatectomy these biomarkers could reduce the likelihood of enrolling high risk men and facilitate safe patient selection for active surveillance.

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Keywords

active surveillance; Gleason grade 3; PTEN; *MYC*/8q; *LPL*/8p

Introduction

Increasing recognition of over-treatment of prostate cancer has stimulated use of active surveillance as a management strategy in select men with low grade, low volume prostate cancer (1, 2). For many active surveillance programs a key eligibility criterion is biopsy Gleason score 6 or less with no Gleason Pattern 4 tumor. However, studies of men with low risk tumors who were eligible for active surveillance but instead opted for immediate prostatectomy have shown that standard 12 core biopsy misses higher grade tumor in 25–35% of cases with only Gleason 6 on biopsy (1, 3, 4). Because active surveillance programs generally perform surveillance (repeat) biopsies on a regular basis, most men under-sampled by the initial biopsy are subsequently detected with higher grade disease and offered curative treatment. Most of these men are found to have Gleason 3+4 tumors at surgery, and have a high probability of cure. However, in 17–32% of these cases Gleason 4+3 or 4+4 (or higher) tumors are found upon prostatectomy, and it is not known if the delay in treatment of such cases compromises the chance of cure (5–7).

Currently, there is a lack of data on biomarkers that consistently show an association with Gleason upgrading from biopsy to radical prostatectomy. We and others have shown that alterations in *PTEN* (protein or copy number alteration), *MYC*/8q, and *LPL*/8p are consistently associated with both higher Gleason score tumors, and increased risk of progression or death after prostatectomy (8–18). In particular, we recently showed that PTEN loss is associated with upgrading from Gleason 6 to 7 (19).

Because of the consistency with which *PTEN* loss and chromosome 8p/8q alterations have been associated with aggressive prostate cancer phenotype, they may have potential for identifying men who appear eligible for active surveillance (i.e. biopsy Gleason 6) but who actually harbor higher grade tumor that was not detected by biopsy. Since active surveillance is most often restricted to men with only Gleason pattern 3 on biopsy, the ideal tissue biomarker would be able to indicate the presence of *unsampled* pattern 4 in the prostate when measured on biopsy cores exhibiting only Gleason pattern 3. Therefore, we evaluated whether measurement of *PTEN* gene or protein, chromosome 8q (*MYC*) gain, 8p (*LPL*) loss in Gleason grade 3 (G3) tumor cores could distinguish those that came from Gleason 3+3=6 vs. Gleason 3+4 or 4+3=7 tumors.

MATERIALS AND METHODS

All biomarker analyses for the primary analyses were measured *only on the G3 component* of Gleason 6 or Gleason 7 tumors.

Study subjects

Records were reviewed retrospectively for men who underwent radical prostatectomy at Johns Hopkins Hospital from 2001–2009 to identify those with available paraffin-embedded

tumor tissue whose prostatectomy specimen exhibited only a single Gleason score, i.e. either Gleason 3+3 only, 3+4 only, or 4+3 only. The protocol was approved by the Johns Hopkins Institutional Review Board. Cases were graded according to ISUP 2005 (20). Year of surgery was restricted to ensure that no specimens had been stored for more than 10 years at the time of biomarker analyses. Tumors from men with Gleason 3+3 were identified and matched by age at surgery (±5 years; median 2 years), year of surgery (±3 years; median 1 year), and race to one case of Gleason 3+4 and one case of Gleason 4+3. Fifty men were selected in each of the 3 Gleason score categories. All tumors had to have adequate tumor volume to accommodate removal of 4 × 0.6 mm tissue cores. Also, cases of 3+3=6 tumor were excluded if there was any tertiary Gleason pattern 4.

Tissue microarray construction

For each patient, four 0.6 mm cores of tumor nodule and four 0.6 mm cores of surrounding benign prostate tissue were manually punched and assembled into a 20 × 20 spot tissue microarray using a Beecher microarrayer as previously described (21). For Gleason 7 tumors cores were sampled from both G3 and G4 areas of tumor (Fig. 1). Matched sets (Gleason 3+3, 3+4, 4=3) were placed on the same tissue microarray block, ensuring that comparison groups were processed identically, thus avoiding bias due to batch effects. In cases with Gleason pattern 4, all cores were obtained from separate areas of Gleason pattern 3 and Gleason pattern 4 from “index” tumors (e.g. the largest and highest grade tumor in the prostate) that contained both patterns. In other words, the Gleason pattern 3 and 4 were spatially adjacent and part of the same overall tumor nodule. Formalin-fixed and paraffin-embedded cell lines with and without targeted disruption of both *PTEN* alleles were used as positive and negative controls for immunohistochemistry (12). Additionally, benign tissues from a variety of organs were also included as positive controls for staining.

PTEN immunohistochemistry

PTEN immunohistochemistry was performed manually as previously described (12) on 4 μm tissue microarray sections using a rabbit monoclonal α-PTEN antibody (clone D4.3, Cell Signaling Technologies). Tissue microarray slides were scanned using the Aperio ScanScope® CS virtual slide scanner (Aperio, Vista, CA) and composite tissue microarray core images were viewed using the TMAJ software package (http://tmaj.pathology.jhmi.edu). Scoring of PTEN expression in tumor cells was performed by 2 pathologists (ADM and BG), blinded with respect to FISH results, pathologic stage, and final Gleason score at radical prostatectomy, as well as patient outcome. PTEN protein was visually scored using a dichotomous system (12, 14), and classified as lost if the intensity was markedly decreased or entirely negative across all tumor cells compared with the surrounding benign glands and/or stroma. Each individual patient was classified as markedly decreased for PTEN if any of his tumor spots were classified as markedly decreased. Cases heterogeneous for PTEN loss, in which some tumor cells stained positive while others showed loss within the same core, were recorded as having PTEN loss if >10% of tumor cells within the core were negative or markedly decreased (12). Inter-observer reproducibility for this scoring system (e.g. diagnosing a given core with PTEN loss or not) has been shown to be excellent (12) and in the current study was 95% for all cores with cancer.

PTEN fluorescence in-situ hybridization (FISH)

From the same tissue microarray blocks used for PTEN protein and chromosome 8 FISH, 5 sections of 5-μm thickness were cut for FISH analysis. Hematoxylin and eosin (H&E) sections were also cut before and after the sections for FISH and reviewed to insure the sections contained cancer and identify regions of interest. FISH analysis was performed on deparaffinized sections using PTEN and centromere 10 probe kits (Abbott Molecular, Des Plaines, IL). Sixty representative nuclei from the invasive tumor were scored by a trained cytogenetic technologist with overall evaluation by one of us (RBJ). Abnormal criteria were established though evaluation of 93 normal prostate biopsies included on the tissue microarrays as well as evaluation of the distribution of signal patterns among prostate cancer biopsies on the arrays. *PTEN* gene loss was defined as PTEN/CEN10 ratio <0.8 and ≥60% of cells enumerated with 0–1 *PTEN* signals. Cases were further categorized as having homozygous *PTEN* deletion if >10% of nuclei had 0 *PTEN* signals. The remaining cases were classified as hemizygous *PTEN* gene deletion. Cases with *PTEN* homozygous and hemizygous gene deletion typically had PTEN/CEN10 ratios <0.60 and >0.60/<0.80, respectively. Some cases had gain or loss of a whole chromosome 10. Gain of chromosome 10 required >30% nuclei with 3 or more PTEN and CEN10 signals. Loss of a whole chromosome 10 required >60% of nuclei with 1 PTEN and CEN10 signals. Rare cases had two PTEN signals and three CEN10 signals. Such cases were classified as hemizygous *PTEN* gene deletion.

Chromosome 8q and 8p alterations (FISH)

The method for *LPL* and *MYC* FISH has been previously described in detail (8). Briefly, dual-probe hybridization was performed on tissue microarrays using a centromere 8 probe [chromosome enumeration probe 8 (CEP8); Abbott Molecular, Des Plaines, IL] together with a locus specific probe. An 8p22 probe (*LPL* gene; Abbott Molecular) and 8q24 probe (*MYC* gene; Abbott Molecular) were the locus specific probes. Abnormal criteria were established through evaluation of normal biopsies included on the tissue microarrays. These normal values were similar to those reported by Tsuchiya (8). The copy number status of 8p22, 8q24, and CEP8 in a tissue microarray biopsy was classified as normal, gain, duplication or loss. A case was classified as normal if <30% of nuclei had 3 or more signals and <60% of nuclei had 0 or 1 signal for all the probes. *LPL* was classified as loss if the LPL/CEP8 ratio was <0.85 or if 60% or more nuclei had 0 or 1 LPL signal. *MYC* was classified as gain if 30% or more nuclei had 3 or more MYC probe signals. For the *MYC* gene duplication category, in addition to the gain criteria, it was necessary that the overall mean MYC/CEP8 ratio be >1.3. If both *LPL* and CEP8 were lost and *MYC* was normal, *MYC* was classified as having relative gene duplication.

Statistical analysis

The primary endpoint was prostatectomy Gleason 6 vs. Gleason 7 (3+4 and 4+3 combined). The primary analyses evaluated whether G3 cores from Gleason 7 tumors were more likely than G3 cores from Gleason 6 tumors to exhibit PTEN protein loss (immunohistochemistry), *PTEN* gene deletion (FISH), *LPL*/8p gene loss or *MYC*/8q gene gain (FISH). For most analyses, *PTEN* hemizygous or homozygous gene deletion were combined, and *MYC*/8q

gain or duplication were combined. Analyses were done with the patient as unit of analysis, i.e. a patient was classified as having the specified biomarker alteration ("high risk" status) if any of his G3 tumor cores exhibited the high risk alteration; otherwise he was classified as "normal" for that biomarker. The sample size was selected to provide power ≥80% to detect a minimum increase of 20% in the prevalence of PTEN protein loss (immunohistochemistry) in Gleason 7 tumors, assuming that the prevalence was ≤10% in Gleason 6 tumors. Descriptive characteristics were compared among Gleason 3+3, 3+4, and 4+3 using analysis of variance with blocking on matched set, or Mantel-Haenszel chi-square test for continuous and categorical variables, respectively. We compared the status of each biomarker to Gleason 7 vs. 6 using conditional logistic regression to accommodate matched sets of GS3+3, 3+4, and 4+3. A secondary analysis compared Gleason 6 separately to Gleason 3+4 and to Gleason 4+3. All analyses were performed using SAS v9.3 (SAS Institute, Cary, NC).

RESULTS

There were 50 men in each of the three Gleason score categories (6, 3+4, 4+3), matched on age (±5 years; median 3 years), and year of surgery (±3 years; median 1 year). Of these, 142 (95%) had at least 1 core of G3 tumor that was informative for all four candidate biomarkers; these 142 men formed the basis for analysis.

The characteristics of men in each of the 3 Gleason score groups are shown in Table 1. As expected, both PSA and prostatectomy stage increased significantly with Gleason score. When evaluated only in G3 cores, PTEN protein loss (by immunohistochemistry), *MYC*/8q gene gain and *LPL*/8p gene loss (both by FISH) increased significantly with Gleason score, but the association with *PTEN* gene deletion was not statistically significant, although deletion appeared more frequent among Gleason 4+3 patients. Fig. 2A shows the trend between expression of each biomarker with increasing Gleason score. One or more high risk biomarker alterations were present in G3 cores from 23% of Gleason 3+3 tumors vs. 63% of Gleason 3+4 tumors vs. 72% of Gleason 4+3 tumors, $p<0.0001$ for trend. If Gleason score was dichotomized to 6 vs. 7 the association with PTEN protein loss, *MYC*/8q gene gain, and *LPL*/8p gene loss remained significant ($p=0.01$, <0.0001, <0.0001, respectively), while the association with *PTEN* gene deletion was not statistically significant ($p=0.538$) (data not shown). None of the four biomarkers were significantly associated with race, age, PSA, PSA density, number of positive biopsy cores, body mass index, or year of surgery (data not shown), with the exception of a significant decrease in age associated with *MYC/8q* gain, $p=0.021$. PTEN protein loss and *LPL/8p* gene loss were significantly associated with worse clinical stage, $p=0.002$ and .006, respectively. PTEN protein loss was significantly correlated with *PTEN* gene deletion, $p<0.0001$, and *LPL*/8p loss, $p=0.001$, but not with *MYC*/8q gain, $p=0.386$ (Table 2).

In univariate logistic regression analyses, PTEN protein loss, *MYC*/8q gene gain, and *LPL*/8p gene loss in a G3 core were associated with a statistically significant 4 to 5-fold increase in the odds that the tumor was Gleason 7 vs. Gleason 6, but *PTEN* gene deletion (hemizygous or homozygous deletion) was not statistically significant (Table 3). In multivariable models *MYC*/8q gain and *LPL*/8p loss, or *MYC*/8q gain and PTEN protein loss were independently predictive (both models are considered because the strong

collinearity between PTEN protein loss and *LPL*/8p gene loss makes it questionable to evaluate both variables in the same model) (22). Adjustment for age, PSA, clinical stage, number of positive biopsy cores, body mass index, or year of surgery did not change the association between biomarkers and prostatectomy Gleason score (data not shown), so only the biomarker results are shown. We also evaluated the impact of having alteration in more than one biomarker. Compared to having no biomarker alterations, having 1, 2, or 3–4 different biomarker alterations in a G3 core was associated with increased likelihood of a Gleason 7 tumor, odds ratio=6.04 (p=.003), 9.26 (p=.002), or 10.04 (p=0.015), respectively (data not shown). Forty-one patients (29%) had 2 or more different biomarker alterations in a G3 core.

In secondary analyses, models were separately constructed with Gleason 6 vs. 3+4, and Gleason 6 vs. 4+3 (Table 4). For all 4 biomarkers, associations were stronger with Gleason 4+3 than with Gleason 3+4. For Gleason 3+4, only the association with *MYC*/8q gain was statistically significant, although all but *PTEN* gene deletion show elevated odds ratios. In contrast, for Gleason 4+3, PTEN protein loss, *MYC*/8q gain, and *LPL*/8p loss all show significant positive associations. We also evaluated whether the likelihood of a Gleason 7 tumor increased with the number of G3 cores or percentage of G3 cores exhibiting biomarker alteration. Using either metric there was a significant association between increased “dose” of cores with *MYC*/8q gain or *LPL*/8p loss and likelihood that the cores came from a Gleason 7 tumor. In contrast, having more than one core with PTEN protein loss did not increase the risk of a Gleason 7 tumor beyond the increase observed for one core (data not shown).

Considering all cores (G3 and G4) from patients with Gleason 7 tumors we evaluated whether biomarker alteration in a G3 core occurred more commonly when the alteration was also present in one of the matched G4 cores. Among tumors with a biomarker alteration in at least 1 G3 core, 65–78% of tumors also had the alteration in at least one of the matching G4 cores (Figure 2B). These data indicate that alterations in these candidate biomarkers in Gleason 7 tumors rarely occur in G3 glands without concomitant alteration in the G4 glands.

DISCUSSION

Most current protocols for active surveillance depend on biopsy pathology as a major determinant of eligibility, with detection of Gleason G4 frequently indicating the need for treatment rather than surveillance. A limitation of these protocols is that needle biopsy underestimates tumor grade in 25–35% of cases, and in such cases, the true grade may require several rounds of surveillance (follow-up) biopsies before it is revealed or it may be missed altogether (23). Until a signature of aggressive phenotype independent of Gleason grade is developed, biomarkers that can indicate if a biopsy G3 is associated with un-sampled G4 tumor would be a major improvement in our ability to safely assign men to active surveillance. In this study we have demonstrated that a G3 core that exhibits PTEN protein loss, *MYC*/8q gene gain or *LPL*/8p gene loss is much more likely to have come from a Gleason 7 than Gleason 6 tumor, and that the association is even stronger for Gleason 4+3 than 3+4 tumors. *PTEN* hemiozygous or homozygous gene deletion appeared to be

somewhat more common in G3 cores from Gleason 7 tumors, but the difference was not statistically significant.

Our findings suggest that occurrence of these biomarker alterations in G3 tumor foci is a strong indicator of the likely presence of adjacent G4 tumor that was not sampled by the biopsy, and thus, a more aggressive phenotype than expected for a purely pattern 3 histology. Furthermore, we observed that, in Gleason 7 tumors, biomarker alteration in G3 glands is much more likely when the matching G4 glands also exhibit the alteration. These findings are consistent with a recent study that sequenced *TMPRSS2* and *ERG* loci from Gleason 3+4 tumors from four patients whose tumors exhibited a *TMPRSS2-ERG* fusion event, and found that adjacent G3 and G4 tumor foci exhibited identical breakpoints, indicating that the two Gleason patterns were clonally related (24). In two cases, there was loss of one *PTEN* allele in both components, yet the Gleason G4 tumor showed loss of both *PTEN* alleles (24). This finding suggested that, at least at times, a Gleason pattern 4 tumor may clonally evolve from the adjacent Gleason pattern 3 lesion or that they both came from a common precursor lesion and *PTEN* gene deletion may be a characteristic of such molecular progression (24). Kovtun et al., also found by next generation sequencing that tumors with mixed G3 and G4 also showed strong evidence of a clonal relationship (25). Our study does not address the question of whether a G3 only lesion that was remotely located in the prostate away from any G4 lesion would also have an increased prevalence of PTEN loss, 8p loss or 8q24 gain. Future studies are required to address this important question.

There has been little evaluation of PTEN as a predictor of aggressive phenotype in patients managed by (or eligible for) active surveillance, and we are not aware of studies that have evaluated *MYC*/8q or *LPL*/8p in such patients. In a Swedish watchful waiting cohort, a signature associated with embryonic stem cells, p53 mutation or inactivation, and PTEN loss was strongly associated with higher Gleason grade, and with a 3-fold increase in risk of death (26). In transurethral resection of the prostate specimens from 675 men managed conservatively, Cuzick *et al.* observed PTEN protein loss was significantly more prevalent in Gleason 7 (20%) than Gleason 6 tumors (3%) (16). Although not managed conservatively, a small series comparing tumors classified as clinically insignificant according to Epstein criteria (i.e. Gleason 6, <3 cores positive, ≤50% of any core involved with tumor; the same criteria used by many institutions to define eligibility for active surveillance) vs. clinically significant tumors (predominantly Gleason ≥7), PTEN protein loss was observed in 0/7 insignificant tumors vs. 8/19 significant tumors (27).

We believe this is the first proof of concept demonstration that biological characteristics of the Gleason pattern 3 component of a Gleason score 7 tumor are distinctly and significantly different than that of a Gleason score 6 tumor, indicating that biopsy Gleason 6 can at times be misleading. The results imply that interrogation of molecular features within a Gleason 6 biopsy can augment the ability of standard Gleason grading by traditional histopathology to predict overall prostate pathology. Indeed, in a recent study, members of our group showed that PTEN protein loss in Gleason 6 biopsies was much more common in tumors that were upgraded to Gleason 7 at prostatectomy compared to tumors that remained Gleason 6 at prostatectomy (19). If replicated in additional studies, these results may stimulate a widespread change in practice to include PTEN immunohistochemistry in apparently low

volume Gleason 6 tumors to help determine appropriateness for active surveillance. Further studies using prostate needle biopsies to measure all 3 biomarkers from the present study are clearly warranted.

It is not clear why *PTEN* gene deletion by FISH did not correlate with increased Gleason score, while PTEN protein loss by immunohistochemistry did. Although *PTEN* gene deletion by FISH did correlate with PTEN protein loss in a highly significant matter ($P<0.0001$), there were still several discrepancies between these, especially at the individual core level. The largest discrepancy was when PTEN protein was markedly decreased by immunohistochemistry but there was no apparent loss by FISH (21 of 58 discrepant spots; 36%). We and others have previously shown that in 30–40% of cases with PTEN immunohistochemistry loss, there is no underlying PTEN gene deletion detected by FISH (12, 28). The simplest explanation is that, while PTEN protein loss in prostate cancer occurs almost always by deletion (and not point mutations for example), the deletions can be quite small and many of these may not be picked up by the relatively large *PTEN* FISH probe used in our assay. It is not clear how our results may differ if we use the newer four-color *PTEN* FISH probes, which are suggested to be more sensitive and specific for finding PTEN deletions than a two color approach (29, 30). Discrepancy between immunohistochemistry and FISH could also arise if assessment of FISH was performed in a different area than was lost by immunohistochemistry. This could result in cases in which PTEN protein loss by immunohistochemistry is heterogeneous in the tumor core, yet is easily recognized; however, if the part of the core that was not lost by immunohistochemistry was counted by FISH, the case would be likely recorded as having no FISH abnormality. In 9 tissue microarray spots, FISH scoring indicated a homozygous deletion yet there was no protein loss recorded by immunohistochemistry. There appeared to be a number of potential reasons for this, perhaps the most important of which is that PTEN immunohistochemistry can be difficult to interpret in a small percentage of cases. Although there was 95% concordance in PTEN calls by different observers in the current study overall, in some cores (including 3/9 of the above-described cores), different observers scored the immunohistochemistry differently, indicating that these cores were difficult to call by immunohistochemistry. In one core, there was a clear decrease in immunohistochemistry staining, however, this was not enough to reach a threshold for calling the core markedly decreased. We have shown previously that the specificity of immunohistochemistry staining using this assay is extremely high (e.g. ≥95% for mutant cell lines (12)) so it is unlikely that a significant fraction of cores would be misclassified as positive staining when indeed there is no PTEN protein present.

The study has a number of strengths, including matching of Gleason 6 and 7 tumors on age, race, and year of surgery, use of well-validated assays, high quality tissue microarray constructed with tumor samples that exhibited only a single Gleason score and were <10 years old, and inclusion of multiple cores from each grade component. However, there are some limitations. First, the biomarkers were assayed in tissue microarray cores taken from prostatectomy specimens, not biopsies. Although targeted sampling of the index tumor by this approach is an imperfect model of the relatively blind sampling conducted with typical transrectal ultrasound biopsies, it may be a reasonable model of the type of targeted sampling that is becoming increasingly available with MRI-guided biopsies. However, it is

clear that additional validation of the joint utility of these biomarkers in a series of biopsies from active surveillance patients is necessary to determine whether biomarker alterations in Gleason 3+3=6 biopsies are more common in patients who are upgraded to Gleason ≥7 during follow-up or surgery. These studies in biopsy specimens are ongoing. Second, since a combination of MRI-ultrasound fusion guided biopsies with standard systematic biopsies increases the ability to identify tumors with Gleason 7 and higher (31–33), future studies employing the biomarkers herein along with MRI-guided biopsies should be performed to determine the relative value of each; in particular, whether these biomarkers add prognostic information beyond Gleason. Third, the sample size of 142 patients is moderate, and only a relatively small percentage of patients exhibited PTEN protein loss (15%), *PTEN* gene deletion (14%), and *MYC*/8q gain (20%). The cohort size and use of only recent cases limited the ability to evaluate clinical outcomes such as biochemical recurrence or metastasis. However, ample data from other studies demonstrate that these biomarker alterations are prognostic for clinical outcomes. Furthermore, associations we observed between biomarker status and grade were not modified by adjustment for other relevant pre-surgical prognostic features.

CONCLUSIONS

PTEN protein loss, *MYC*/8q gain, or *LPL*/8p loss in a G3 tumor core is a strong indicator that the core comes from a Gleason 7 tumor, and occurs even more frequently in G3 cores from Gleason 4+3 than 3+4. Among Gleason 7 tumors, the predominance of these biomarker alterations in both the G3 and G4 cores provides additional evidence that such tumors may be clonally related in many cases. Further, the results suggest that histological Gleason pattern 3 sampled from a Gleason 7 cancer is often biologically distinct from Gleason pattern 3 from a Gleason 6 tumor. Combined with recent data showing that PTEN protein loss is more common in Gleason 6 tumors that are upgraded at prostatectomy (11, 19, 30), and, showing that a PTEN immunohistochemistry assay based on our highly validated approach (12) can now be implemented readily in CLIA certified pathology laboratories using automated staining systems with a commercial anti-PTEN antibody (18) these results suggest that these biomarkers may have significant clinical utility for identifying men who are not suitable candidates for active surveillance.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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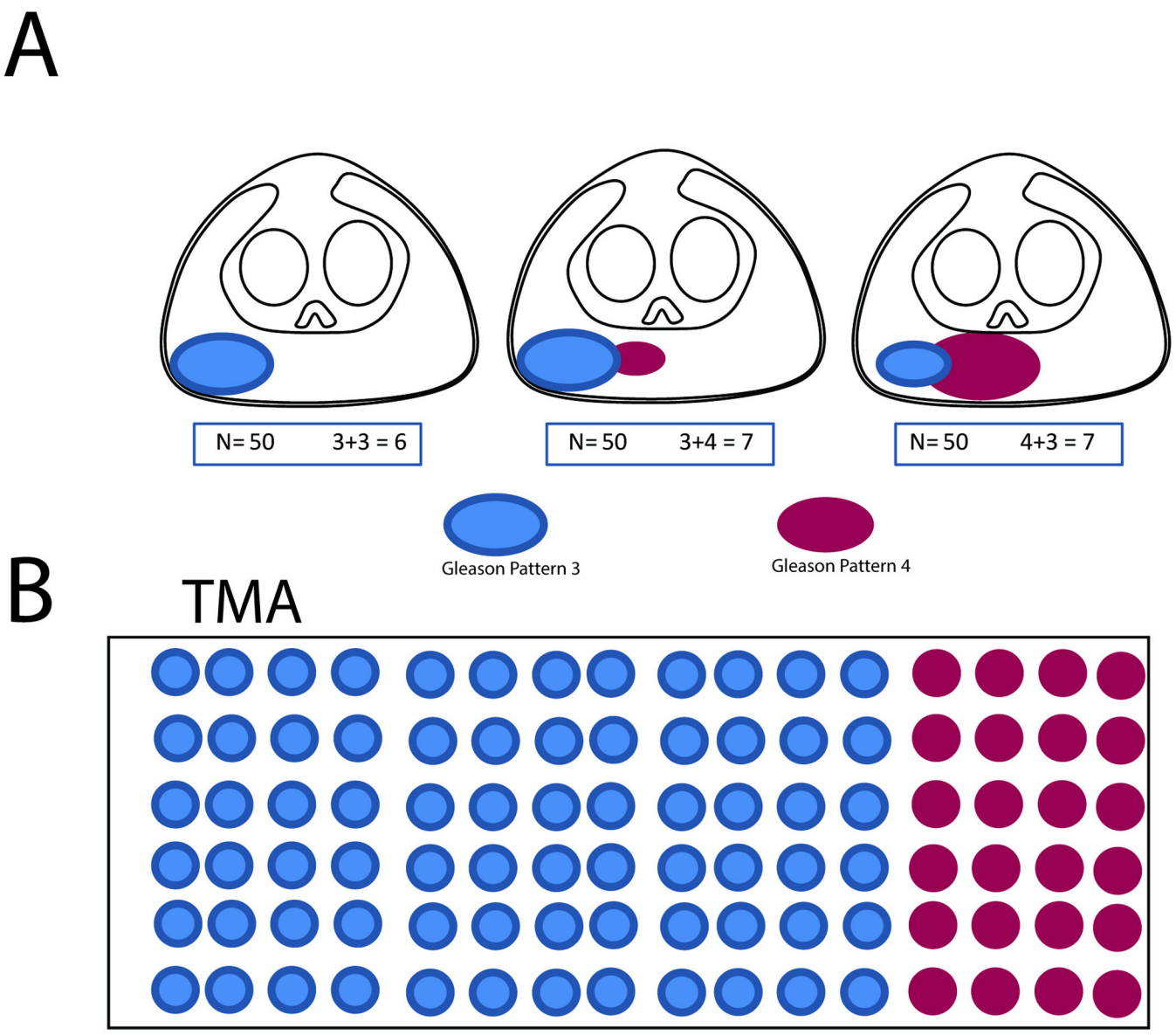

Figure 1.

A, Sampling scheme to select Gleason pattern 3 and Gleason pattern 4 tumor cores from matched cases of Gleason score 3+3, 3+4, and 4+3 for tissue microarray construction. B, Schematic of tissue microarray construction. Note that 4 cores from each Gleason pattern were selected for the tissue microarray.

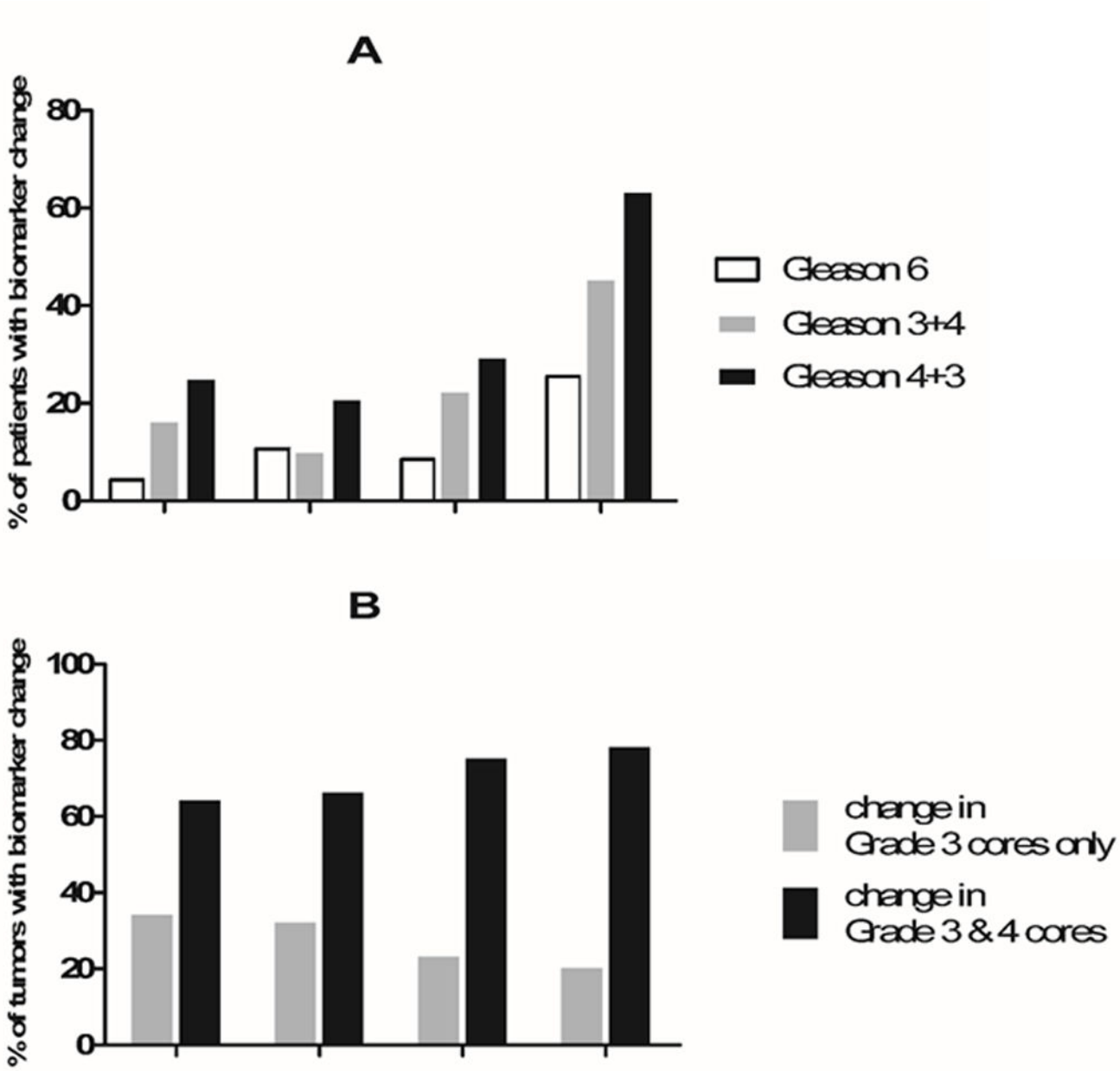

Figure 2.

A, Frequency of PTEN protein loss, *PTEN* gene deletion, *MYC*/8q gene gain, and *LPL*/8p gene loss in Gleason pattern 3 cores from matched Gleason score 3+3, 3+4, and 4+3 tumors (n=142). B, Frequency of biomarker alteration in a grade 3 core is associated with biomarker alteration in at least 1 matched grade 4 core in Gleason score 7 tumors.

* Number of tumors with indicated biomarker alteration in a grade 3 core.

Table 1

Characteristics of study participants, by prostatectomy Gleason score

	Gleason Score			
Characteristic (n)	**6 (47)**	**3+4 (48)**	**4+3 (47)**	**p-value**
Age, median (years)	61.0	60.0	60.0	0.746 *
Year of surgery, median	2005	2005.5	2005	0.590 *
Race, n (%)				1.000 **
Caucasian	46 (98)	47 (98)	46 (98)	
African-American	1 (2)	1 (2)	1 (2)	
PSA, median (ng/ml)	4.4	5.1	6.5	0.0002 *
Prostatectomy stage, n (%)				<0.0001 **
organ-confined	40 (85)	29 (60)	20 (43)	
EPE	7 (15)	19 (40)	19 (40)	
SVI	0 (0)	0 (0)	4 (9)	
LNI	0 (0)	0 (0)	4 (9)	
Surgical margin status, n (%)				0.242 **
Negative	41 (93)	36 (80)	38 (84)	
Positive	3 (7)	9 (20)	7 (16)	
PTEN immunohistochemistry ***, n (%)				0.005 **
Not decreased	45 (96)	40 (83)	35 (75)	
Markedly decreased	2 (4)	8 (17)	12 (26)	
PTEN FISH ***, n (%)				0.140
Normal	42 (89)	43 (90)	37 (79)	
Hemizygous deletion	1 (2)	2 (4)	5 (11)	
Homozygous deletion	4 (9)	3 (6)	5 (11)	
MYC/8q FISH ***, n (%)				0.011 **
Normal/loss	43 (92)	37 (77)	33 (70)	
Gain/duplication	4 (9)	11 (23)	14 (30)	
LPL/8p *** FISH, n (%)				0.0002 **
Normal/duplication	35 (75)	26 (54)	17 (36)	
Loss	12 (26)	22 (46)	30 (64)	

Abbreviations: EPE, extra-prostatic extension; SVI, seminal vesicle involvement; LNI, lymph node involvement; FISH, fluorescence in-situ hybridization

* based on Kruskal-Wallis test

** based on Mantel-Haenszel chi-square test

*** Biomarker status based on Gleason grade 3 core only

Table 2

Association between PTEN loss by immunohistochemistry and PTEN deletion, 8q/*MYC* gain, and 8p/*LPL* loss by FISH * among Gleason grade 3 cores from prostate tumors with Gleason score 6 or 7 (n=142).

	PTEN (FISH)		**8q/*MYC* (FISH)**		**8p/*LPL* (FISH)**	
PTEN (immunohistochemistry)	**normal**	**deletion**	**normal**	**gain/dup**	**normal**	**loss**
normal # (proportion)	112 (0.93)	8 (0.07)	97 (0.81)	23 (0.19)	73 (0.61)	47 (0.39)
decrease # (proportion)	10 (0.45)	12 (0.55)	16 (0.73)	6 (0.27)	5 (0.23)	17 (0.77)
p-value	<0.0001		0.386		0.001	

* A patient was considered to have the specific biomarker alteration if any grade 3 core exhibited the change

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Table 3

Conditional logistic regression models of PTEN loss by immunohistochemistry, and *PTEN* deletion, 8q/*MYC* gain, and 8p/*LPL* loss by FISH* in grade 3 cores as predictors of Gleason score 7 vs. 6 (n=142)

Univariate Models		
Biomarker Alteration	**Odds Ratio (95% CI)**	**p-value**
PTEN loss vs. normal (immunohistochemistry)	4.99 (1.14, 21.98)	0.033
PTEN deletion vs. normal (FISH)	1.44 (0.50, 4.18)	0.498
MYC/8q gain vs. normal (FISH)	5.36 (1.50, 19.09)	0.010
LPL/8p loss vs. normal (FISH)	3.96 (1.58, 9.94)	0.003

Multivariable Models		
Biomarker Alteration	**Odds Ratio (95% CI)**	**p-value**
MODEL 1		
PTEN loss vs. normal (immunohistochemistry)	5.21 (1.14, 23.76)	0.033
MYC/8q gain vs. normal (FISH)	5.78 (1.51, 22.06)	0.010
MODEL 2		
MYC/8q gain vs. normal (FISH)	5.28 (1.35, 20.58)	0.017
LPL/8p loss vs. normal (FISH)	3.78 (1.46, 9.80)	0.006

Abbreviations: FISH, fluorescence in-situ hybridization; OR, odds ratio; CI, confidence interval.

*A patient was considered to have the specific biomarker alteration if any grade 3 core exhibited the change

Table 4

Univariate logistic regression models of PTEN loss by immunohistochemistry, and *PTEN* deletion, 8q/*MYC* gain, and 8p/*LPL* loss by FISH* in grade 3 cores as predictors of Gleason score 3+4 vs. 6 (n=95), and Gleason score 4+3 vs. 6 (n=94)

	Gleason 3+4 vs. 6		Gleason 4+3 vs. 6	
Predictor variable	**OR (95% CI)**	**p-value**	**OR (95% CI)**	**p-value**
PTEN loss vs. normal	3.50 (0.73, 16.85)	0.118	5.50 (1.22, 24.80)	0.027
PTEN deletion** vs. normal	0.80 (0.22, 2.98)	0.739	2.67 (0.71, 10.05)	0.147
MYC gain vs. normal	8.00 (1.001, 63.96)	0.0499	6.00 (1.34, 26.81)	0.019
LPL loss vs. normal	2.60 (0.93, 7.29)	0.069	9.00 (2.09, 38.79)	0.003

Abbreviations: FISH, fluorescence in-situ hybridization; OR, odds ratio; CI, confidence interval.

*A patient was considered to have the specific biomarker alteration if any grade 3 core exhibited the change